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Phytochemistry and GCMS Analysis of Ethanol and Aqueous Stembark Extracts of *Detarium microcarpum* Guill. & Perr. Fabaceae

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Abstract: The therapeutic applications of medicinal plants in the treatment of various diseases can be attributed to their diverse phytochemical constituents. This study aimed to investigate the phytochemical composition of aqueous and ethanol stem bark extracts of *Detarium microcarpum*. Qualitative and quantitative analyses were conducted to determine the presence and concentrations of phytochemicals, followed by the identification of phytoconstituents using gas chromatography-mass spectrometry (GC-MS). The aqueous extract was found to contain saponins (27.11 ± 0.22%) and flavonoids (47.33 ± 0.70%), with alkaloids, steroids, glycosides, and terpenoids absent. In contrast, the ethanol extract contained alkaloids (10.78 ± 0.59%), saponins (45.11 ± 0.48%), glycosides (5.44 ± 0.48%), and flavonoids (11.00 ± 0.77%), while steroids and terpenoids were not detected. GC-MS analysis revealed 14 compounds in the aqueous extract and 20 in the ethanol extract. The major constituents of the aqueous extract included hydroperoxide, 1,4-dioxan-2-yl (58.32%), 1,2,3-benzenetriol (16.44%), and cis-p-coumaric acid (11.05%). In the ethanol extract, the predominant compounds were coumarin (29.41%), benzofuran (17.23%), and catechol (9.23%). The identified compounds may serve as potential synthetic templates for the development of novel therapeutic agents targeting various diseases. This study supports the ethnomedicinal use of *D. microcarpum* and provides a scientific basis for its role in traditional medical practices.

Introduction

The phytochemical composition of medicinal plants has been the reason for their extensive employment in ethnomedicine. These phytochemicals constitute different bioactive compounds that are attributed to the pharmacological and biological activities of the different plant parts. In traditional practice, different parts of plants are prepared in different formulations; maceration, decoctions, and infusions, administered to achieve therapeutic goals (1). Medicinal plants exhibit efficacy in phytotherapy of various ailments using different plant parts (2-7). Poor and ineffective medical care and the cost of drugs especially in developing countries have been the driving force for the prospect of medicinal plants, thus affordable alternative (8). Over 100,000 different kinds of plants are associated with various pharmacological and biological activities attributed to the phytoconstituents of the plants (9-12).

Detarium microcarpum (*D. microcarpum*) tree is native to Africa and grows in Central and West African regions (13). The plants are called tallow three in English and Taura by the native Hausa people in Nigeria. This plant is often applied in ethnomedicine to treat diseases due to the pharmacological activities associated with the plant including the phytotherapy of diarrhea, meningitis, tuberculosis, hemorrhoids, and diabetes (14, 15). *D. microcarpum* comprises high phenolic compounds with free radical scavenging capabilities, acting as an antioxidant for oxidative stress-linked diseases (16). The antimicrobial activity of *D. microcarpum* against *Streptococcus aureus and Escherichia coli* was attributed to their phytochemical composition (17, 18). In another report, the

antibacterial activity of *D. microcarpum* against *salmonella thyphimurium* supported its application in the phytotherapy of typhoid infections (19). Antimalarial activity of *D. microcarpum* was previously reported in infected mice and an assessment of the hematological and biochemical parameters revealed the safety of the plants (20).

A previous study reported various pharmacological activities of different parts of *D.* microcarpum, including hypoglycemic, antimicrobial, anti-parasitic, and anti-inflammatory properties (15, 18). Furthermore, the plant was reported to be a source of novel drugs attributed to its biological activities of the plant against Alzheimer's and snake venoms (21). In another study, *D. microcarpum* was reported to possess anticancer activity evident to its cytotoxicity which was linked to its application in ethnomedicine and potential for the development of novel anticancer drugs (22). Moreover, it has been traditionally employed in diabetic therapy in most regions of Africa (23). An ethnobotanical survey of traditionally used plants for the management of cancer reported the use of the root and stembark *of D. microcarpum* in Borno State, Nigeria (24). The plant of study has been reported to have different pharmacological activities, attributed to their phytochemical composition (25). Thus, this study aimed to examine the phytochemical profile of the aqueous and ethanol stembark extracts of *D. microcarpum* due to its extensive use in traditional ethnomedicine.

Methodology

Materials

The stembark of *D. microcarpum* was obtained from Girei Local Government Area of Adamawa state, Nigeria. It was authenticated by a Forest Technologist from the Forestry Department, Adamawa State Polytechnic Yola, where a voucher specimen with voucher number ASP/FT/0099 was deposited. The stembark was air-dried and ground into powder using a blender.

Extract Preparation

D. microcarpum stembark powder (300 g) was macerated in 1 L of distilled water and ethanol separately in glass jars for 48 h. The mixtures were separately filtered using a Whatman no. 1 filter paper, followed by concentrating the filtrate to dryness in an oven at 40 °C to yield 21 and 34 g of aqueous (ASBE) and ethanol (ESBE) stembark of extracts *D. microcarpum*, respectively (26).

Phytochemical Screening and Quantitation

Phytochemical identification of the ASBE and ESBE was carried out by the methods described previously by Evans (26) to detect the presence of alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids.

Total Alkaloids

Alkaloids were separately quantified from each extract as previously described by Harborne (27). Exactly 10 mL of 10% ammonium hydroxide was added to 0.2 g of the ASBE and ESBE, separately to convert alkaloidal salts into free bases, followed by stirring. The mixtures were filtered after 4 h and concentrated over a water bath, followed by a drop-wise addition of concentrated ammonium hydroxide to precipitate the alkaloids. The precipitate was filtered using an initially weighted filter paper and washed with 10% ammonium hydroxide solution. The filter paper was dried at 60 °C for 30 min and reweighed to a constant weight. The total alkaloids were determined using **Equation 1**. All procedures were carried out in triplicates and presented as a mean of triplicate determinations.

$$Total\ Metabolite = \frac{W_{residue}}{W_{sample}} \times 100\% \quad \textbf{Equation 1}$$

Total Saponins

Saponins were separately quantified by the method previously described by Obadoni et al., (28). Exactly 10 mL of 20% aqueous ethanol was added to 0.2 g of the ASBE and ESBE separately, followed by heating and stirring over a water bath at 55 °C for 1 h. The mixtures were transferred to separating funnels, followed by the addition of 5 mL of diethyl ether and vigorous shaking for proper partitioning. The aqueous layer was collected, followed by the addition of 10 mL of *n*-butanol and 2 mL of 5% NaCl. The mixtures were dried in an oven at 60 °C. The total saponins were determined from **Equation 1**. All procedures were carried out in triplicates and presented as a mean of triplicate determinations.

Total Glycosides

Glycosides were quantified as described previously by Ugwoke et al., (29). Exactly 10 mL of 70% aqueous ethanol was added to 0.2 g of the ASBE and ESBE, separately and boiled for 2 min in a water bath, followed by filtration. The filtrates were diluted with 20 mL of distilled water, followed by the addition of 2 mL of 10% lead acetate and filtration. Furthermore, 10 mL of chloroform was added to the filtrates in a separating funnel and vigorously shaken. The chloroform layer was collected, dried, and weighed. The total glycosides were determined from **Equation 1**. All procedures were carried out in triplicates and presented as a mean of triplicate determinations.

Total Flavonoids

Flavonoids were quantified as previously described by Harborne (27). Exactly 10 mL of 80% aqueous methanol was added to 0.2 g of the ASBE and ESBE, separately. The whole solution was filtered and

evaporated to dryness over a water bath and weighed. The total flavonoids were determined from **Equation 1**. All procedures were carried out in triplicates and presented as a mean of triplicate determinations.

Gas Chromatography-Mass Spectrometric (GCMS) Analysis

The identification of the compounds in the ASBE and ESBE was done with a gas-chromatography mass spectrophotometer system (Model 7890-5975, Agilent, USA) fitted with a fused silica column. The settings and procedure were as previously described (30-33, 12).

Statistical Analysis

The data obtained were expressed as ± standard error of the mean (± SEM) of triplicate determinations. The data were statistically evaluated using Statistical Package for the Social Sciences (SPSS) software (version 22).

Result

Phytochemical Screening and Quantitation

The phytochemicals detected in the ASBE and ESBE of *D. microcarpum* are depicted in **Table 1**. Although alkaloids and glycosides were absent in the ASBE, they were detected in the ESBE. However, both saponins and flavonoids were detected in both extracts. Saponins and flavonoids were detected in the ASBE in concentrations of 27.11 ± 0.22% and 47.33 ± 0.70%, respectively. In the ESBE, saponins had the highest concentration (45.11 ± 0.48%), followed by flavonoids (11.00 ± 0.77%) and alkaloids (10.78 ± 0.59%) while glycosides had the least concentration (5.44 ± 0.48%).

Table 1. Phytochemical composition of the ASBE and ESBE of *D. microcarpum*.

Phytochemical	ASBE	ESBE
Alkaloids	-	10.78 ±0.59
Saponins	27.11 ±0.22	45.11 ±0.48
Steroids	-	-
Glycosides	-	5.44 ±0.48
Terpenoids	-	-
Flavonoids	47.33 ±0.70	11.00 ±0.77
Note: Negative (-) means absent		

GCMS Analysis

The phytochemical analysis of the aqueous stem bark extract (ASBE) of *D. microcarpum* identified a total of 14 distinct compounds, as shown in **Table 2**. Among these, 1,4-dioxanyl hydroperoxide was the most abundant, comprising 58.32% of the extract, followed by 1,2,3-benzenetriol at 16.44%, and cis-p-coumaric acid at 11.05%. Other notable compounds included coumarin, with a peak area of 5.26%, and catechol, contributing 1.99% of the total composition. The chemical structures of the identified compounds are presented in **Figure 1**, highlighting the functional groups and structural features characteristic of each compound. The chromatographic profile of the ASBE, displayed in **Figure 2**, provides a detailed visualization of the retention times and peak areas of the identified compounds. The chromatogram illustrates the prominence of 1,4-dioxanyl hydroperoxide as the major peak, along with secondary peaks corresponding to 1,2,3-benzenetriol and cis-p-coumaric acid. These data underscore the chemical complexity of the ASBE and the dominance of specific bioactive components within the extract.

Table 2. Compounds Identified in the ASBE of *D. microcarpum*.

S/N	Name of compound	Retention Time	Peak Area (%)	Molecular weight	Formula
1	Catechol	3.99	1.99	110.11	$C_6H_6O_2$
2	Coumarin	4.68	5.26	146.15	$C_9H_6O_2$
3	Cis-p-Coumaric acid	5.43	11.05	164.16	$C_9H_8O_3$
4	1,2,3-Benzenetriol	5.75	16.44	126.11	$C_6H_6O_3$
5	1,2,4-Benzenetriol	6.81	1.01	126.11	$C_6H_6O_3$
6	N-Serylserine	6.99	2.27	192.17	$C_6H_{12}N_2O_5$
7	1,4-Dioxanyl hydroperoxide	7.82	58.32	120.11	$C_4H_8O_4$
8	Erucic acid	10.74	1.07	338.57	$C_{22}H_{42}O_2$
9	Trans-13-Octadecenoic acid	11.91	0.50	282.47	$C_{18}H_{34}O_2$
10	cis-11-Hexadecenal	12.30	0.07	238.41	$C_{16}H_{30}O$
11	cis-Vaccenic acid	14.05	0.52	282.47	$C_{18}H_{34}O_2$
12	Oleic Acid	14.52	0.47	282.47	$C_{18}H_{34}O_2$
13	Petroselinic acid	14.74	0.25	282.47	$C_{18}H_{34}O_2$
14	Tetradecanal	15.64	0.77	212.38	$C_{14}H_{28}O$

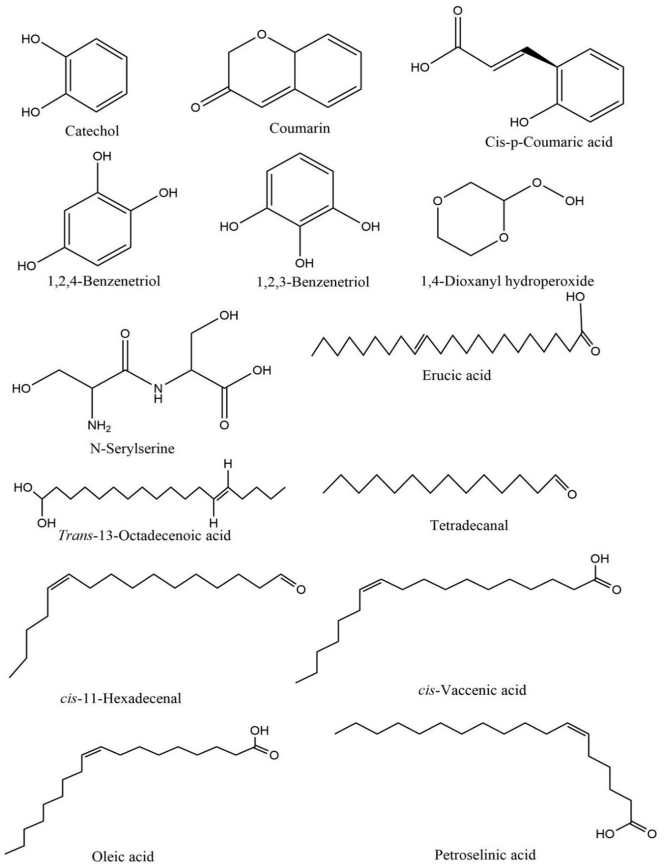

Figure 1. Structures of compounds identified in ASBE of *D. microcarpum*.

Table 3. Compounds Identified in the ESBE of *D. microcarpum.*

S/N	Compound	Retention Time	Peak Area (%)	Molecular weight	Formula
1	Catechol	4.019	9.23	110.11244	$C_6H_6O_2$
2	Coumarin	4.620	29.41	146.14544	$C_9H_6O_2$
3	Benzofuran	5.919	17.23	118.13504	C_8H_6O
4	Methyl palmitate	6.978	8.59	270.45576	$C_{17}H_{34}O_2$
5	1,2,4-Benzenetriol	7.493	4.76	126.11184	$C_6H_6O_3$
6	3-Methyl-1H-pyrazole-5-carboxylic acid	7.807	5.86	126.11484	$C_5H_6N_2O_2$
7	1,2,3-Benzenetriol	8.431	3.87	126.11184	$C_6H_6O_3$
8	1,3,5-Benzenetriol	8.614	2.57	126.11184	$C_6H_6O_3$
9	Phenol, 2-[(1-methylpropyl)thio]-	8.929	2.24	182.28056	$C_{10}H_{14}OS$
10	cis-Vaccenic acid	9.129	1.82	282.46676	$C_{18}H_{34}O_2$
11	Methyl cis-7-hexadecenoate	10.033	0.49	268.43988	$C_{17}H_{32}O_2$
12	2,6,6-trimethylnorpinane	10.256	0.32	138.25292	$C_{10}H_{18}$
13	Octadecanal	10.760	0.23	268.48324	$C_{18}H_{36}O$
14	2,6,10-Trimethylundeca-1,5,9-triene	11.933	1.01	192.34456	$C_{14}H_{24}$
15	Trans-Farnesol	12.328	0.23	222.37084	$C_{15}H_{26}O$
16	9-Tetradecenal, (Z)-	13.856	3.63	210.35984	$C_{14}H_{26}O$
17	Trans-13-Octadecenoic acid	14.050	1.63	282.46676	$C_{18}H_{34}O_2$
18	Tetradecanal	14.474	2.80	212.37572	$C_{14}H_{28}O$
19	Oleic Acid	14.674	1.08	282.46676	$C_{18}H_{34}O_2$
20	Petroselinic acid	15.612	3.00	282.46676	$C_{18}H_{34}O_2$

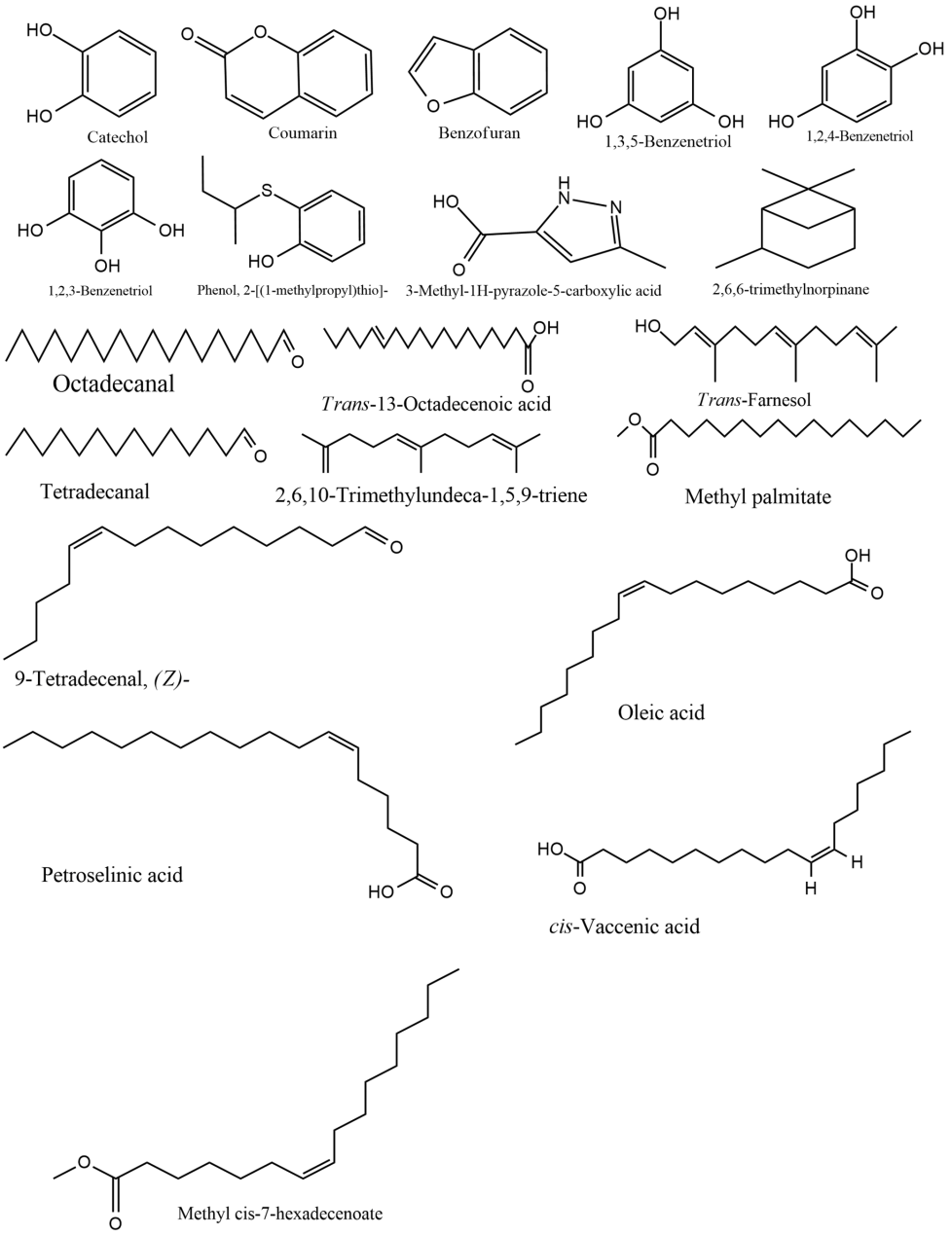

Figure 2. Structures of compounds identified in ESBE of *D. microcarpum*.

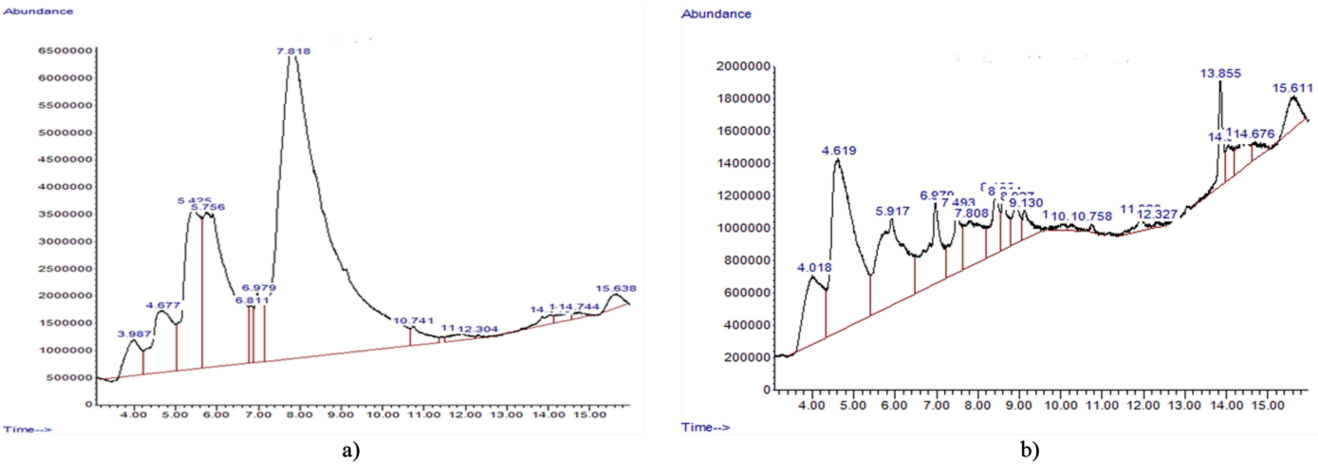

Figure 3. GS-MS Chromatogram of compounds identified in the; a) ESBE and b) ASBE of *D. microcarpum*.

Table 3 shows the compounds identified in the ESBE of *D. microcarpum*. The GCMS analysis identified 20 compounds in ESBE with coumarin being the most abundant (29.41%), followed by benzofuran (17.23%) and catechol (9.23%). The structures of the various compounds identified in the ESBE are presented in **Figure 2**, showing the different groups in the structures while **Figure 3** shows the chromatogram of the ESBE, displaying the peak areas and retention times of the compounds identified.

Discussion

The phytochemicals present in the ASBE and ESBE of *D. microcarpum* are shown in **Table 1**. The absence of some phytochemicals in the aqueous extract though present in the ethanol extract might be due to the difference in solvent polarity as water is more polar than ethanol, thus less hydrophilic phytochemicals might be detected in the ethanol but not the aqueous extract (34). Phytochemicals found in plants have been attributed with different pharmacological roles which include antimicrobial, anti-ulcer, anti-diabetic, antioxidant, and anti-inflammatory activities through different mechanisms of action (35-37, 18, 38, 11, 39, 6, 33, 40). The alkaloid trigonelline extracted from *Allium sepapea, Coffea* sp, *and Pissum sativum* were reported to have several biological activities which include cardio-protective, hepato-protective, hypoglycemic, hypocholesterolemic, and anti-cancers activities (41). In another study, Ding *et al*., (42) reported alkaloids to be associated with antitumor effects and implicated in activities against microbes and inflammation. Mondal *et al*., (43) linked alkaloids to cancer phytotherapy and might serve as a good source for the development of anti-cancer therapeutics. Kohnen-Johannsen *et al*., (44) reported scopolamine (hyoscine) which is another alkaloid as a therapy for nausea and vomiting conditions.

Zhao *et al*., (45) that reported saponins possessed anti-tumor activities through multiple signaling-related pathways, subsequently leading to inhibition of proliferation, promotion of apoptosis, and regulation of tumor microenvironment. Several studies have reported plants having a high amount of saponins in association with cardioprotective pharmacological activities (46-48). Saponins raised the activity of several antioxidant enzymes responsible for cardiac protection against reactive oxygen species (46, 49-52). A saponin (trillin) acted through the anti-lipase mechanism to improve lipid profile by improving lipid peroxidation and superoxide dismutase activity (50). Yang *et al*., (53) reported dioscin as an active agent against a tumor, microbes, inflammation, and oxidative stress.

A Glycoside (phenylethanoid) isolated from natural sources was implicated in the therapy of tumors, inflammation, and microbial infections (54, 55). Glycosides from plant sources also possess anticancer properties, mediated through multiple mechanisms (56). Flavonoids exhibit hepatoprotective and antifibrotic activities, antimicrobial, antidiabetic, and anti-inflammatory activities (57). Flavonoids have been shown to exert effects against *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* (58). The anticancer activity of flavonoids against human hepatocarcinoma by inducing apoptosis through the mitochondria-dependent apoptotic pathway has been previously reported by Hu *et al*., (59). The flavonoid quercetin exhibits anticancer and antiviral application in the management of allergy, inflammation, cardiovascular diseases, and arthritis and potential against Alzheimer's disease which is credited to its ability to inhibit acetylcholinesterase (60). A similar report on the methanol extract of *D. microcarpu*m where alkaloids, saponins, glycoside, and flavonoids were detected agreed with our study (61). In another study, Abdullahi *et al*., (20) reported alkaloids, saponins, glycosides, and flavonoids in the alcohol extract of *D. microcarpu*m. The present study obtained a similar result. A similar study on ethanol extract detected alkaloids, saponins, glycosides, and flavonoids (62) which agrees with the present study.

GCMS analysis revealed 14 compounds in the ASBE of the plant of study (**Table 2**). The compounds identified were aromatic compounds containing hydroxyl groups and long-chain aliphatic fatty acids. Coumaric acid is linked with several biological activities, including anti-cancer, antiviral, and antimicrobial activities. It is also implicated in the management of oxidative stress, inflammation, and arthritis with analgesic properties (63). A previous study demonstrated the antioxidant capacity of coumaric acid (64). In another study, coumaric acid demonstrated anti-fungal activity by retarding the growth of *Fusarium oxysporum* and *Fusarium verticillioides* (65). Anti-bacterial activity of coumaric acid against *Streptococcus pneumonia, Staphylococcus aureus*, and *Bacillus subtilis* through increased membrane permeability and binding to DNA was previously reported (66). Coumaric acid was previously associated with pharmacological activities against hepatitis C through impairment of entry and translation of the virus (67). Anti-viral activity of coumaric acid against the influenza virus by increasing the survival time was reported previously (68). Coumaric acid prevents mutagenesis caused by peroxide radicals which cause aberrations in chromosomes by scavenging the free radicals (69). The antidiabetic of coumaric acid was reported to be through stimulation of insulin secretion, decreased intestinal absorption of carbohydrates, and increased beta-cell activities (70). In a recent study, coumaric acids showed hepato-protective properties induced by carbon tetrachloride

or bile duct ligation and exhibited amoebostatic activity against *Entamoeba histolytica* (71).

1,2,3-Benzenetriol otherwise called pyrogallol identified in both aqueous and ethanol extracts is associated with antibacterial activities against *Staphylococcus aureus* (72). The anti-malarial potential of pyrogallol investigated revealed that the compound can be a candidate for use against malarial parasites due to its auto-oxidation in the presence of metallic ions (Cu^{2+}, Fe^{3+}, and Mn^{2+}) to produce free radicals, thus inhibiting the growth of parasite, a characteristic of anti-malarial drugs (72-74). Anti-bacterial study of pyrogallol reported inhibition of two strains of *Staphylococcus aureus* (75). Coumarin identified was identified in both aqueous and ethanol extracts in the present study have been linked with various pharmacological activities against fungi, bacteria, cancer, and inflammation. It acts as an anticoagulant and antioxidant and also has hypoglycemic and neuroprotective properties (76). The coumarin glycerol possesses anticancer properties through induction of apoptosis (77). Another coumarin butyrate demonstrated anticancer activity by causing apoptosis and inhibiting cancer cells of the colon (78). In an *in vitro* study, glycycoumarin demonstrated anticancer activity against hepatic cancer cells *via* apoptosis induction and shrinking the tumors (79). Glycycoumarin has also been linked with pharmacological activities which include antimicrobial, hepatoprotective, anti-inflammatory, antispasmodic, neuroprotective, antioxidant, and antiviral activities (80).

In the ethanol extract, 20 compounds were identified (**Table 3**). Some of these compounds were reported to be associated with various pharmacological activities. Benzofurans exert antimicrobial activities against fungi and bacteria and act as an anti-tumor anti-breast cancer agent (81). Catechol isolated from *Semecarpus anacardium* seeds was reported to have antioxidant and hypoglycemic activities (82). Methyl palmitate was previously reported to exert some pharmacological activities against inflammation in rats (83). In another study, methyl palmitate demonstrated pharmacological activity by decreasing inflammation through the reduction of the expression of cytokines, promoting inflammation, and increasing the expression of anti-inflammatory cytokines (84). Methyl palmitate shows antioxidant activity by reducing markers of oxidative stress and elevating the activities of the innate antioxidants (84). Methyl palmitate has been previously linked with insecticidal activity (85). Results similar to our study on GCMS analysis of *D. microcarpum* were previously obtained (86, 87).

Conclusion

The results of our study show that different phytochemicals are present in *D. microcarpum* which were previously linked to various pharmacological and biological activities. The compounds identified might be good synthetic sources of novel drugs against different ailments. Thus, this study justified the claims for the extensive use of *D. microcarpum* in traditional ethnomedicine. Additionally, further studies to isolate and determine the pharmacological activities of the compounds reported in our study are warranted.

Abbreviations

GCMS = Gas Chromatography-Spectrophotometry; ASBE = Aqueous Stembark Extract; ESBE = Ethanol Stembark Extract.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

Not applicable.

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